MICRO-MAGNETIC RESONANCE IMAGING OF LIMB DEVELOPMENT: INSIGHTS INTO THE BASIS OF CLUBFOOT

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Introduction: Congenital talipes equinovarus (CTEV), often referred to as "clubfoot", is a malformation immediately recognisable at birth; in which the ankle is in a plantar flexed position, the heel is inverted and the mid-foot and forefoot are inverted and adducted. Prevalence of CTEV varies around the world from 0.3 to 7 per 1000 live births and it may be bilateral or unilateral. Various aetiopathogenic mechanisms for CTEV have been proposed but no evidence has been published to support of them. In order to increase our knowledge of the origins of CTEV, the peroneal muscle atrophy (*pma*) mouse mutant was studied. This is a naturally occurring mouse mutant that has a clubfoot-like malformation in its hind limbs but no other developmental abnormalities. We used μMRI to characterise the three dimensional (3D) musculoskeletal anatomy of mice hind limbs using μMRI and then compared the anatomy of the *pma* and wild type mice hind limbs. We also studied embryonic foot rotation in the hind limbs of normal mice embryos and *pma* mutants to gain insights into the developmental basis of the "clubfoot" phenotype in these mice.

Method: Wild type mouse strains, CD-1 and C57/Black, and the mutant strain *pma* were used. The hind limbs were removed from 3 week-old animals at the level of the proximal femur and fixed. These were imaged on a Bruker Avance FT NMR spectrometer with a wide bore 7.1 T magnet resonating at 300.15 MHz for ^{1}H, fitted with Bruker micro-imaging magnetic field gradients. μMRI TR/TE = 1000/40 ms, T_R/T_E = 200/10 ms and TR/TE = 1000/6 ms spin-echo and T_R/T_E = 250/2.3 ms gradient-echo imaging pulse sequences were acquired. The matrix size varied from 128 x 128 x 128 to 256 x 256 x 256. The field of view ranged from 10-25 mm and voxel dimensions ranged between 78-97 μm. MRI data were Fourier transformed, and then visualised using Amira imaging software and regions of interest segmented and data reconstructed to produce 3D rendered surfaces.

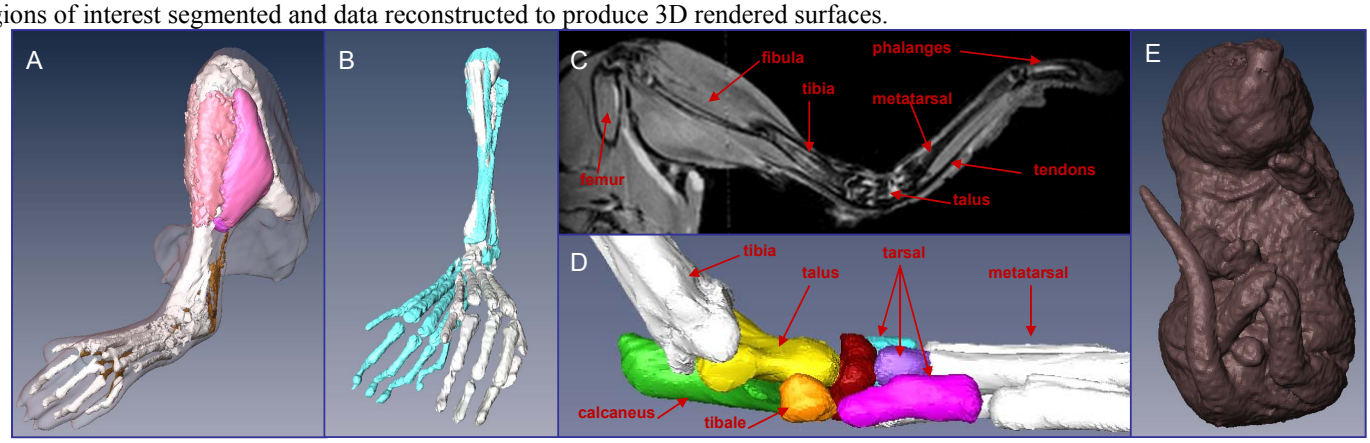

Figure 1: μMRI of 3 week old wild type and *pma* mice left hind legs, (A) 3D surface reconstruction of outer surface, mineralized bones, leg muscles and tendons of *pma* mouse; (B) Overlay of surface reconstructions of mineralized wild type (white) and *pma* (blue) hind leg bones; (C) TR/TE = 1000/40 ms spin-echo sagittal image of wild type hind leg; (D) 3D surface reconstruction of *pma* hind-foot bones. (E) 3D surface reconstruction of day 15 mouse embryo.

Results and Discussion: The musculoskeletal anatomy (Fig.1C) of the wild type and *pma* hind limbs was visualised in 3D using μMRI (Fig.1A). Size, shape and orientation of tibia and fibula, and size and shape of the tarsal and metatarsal bones are similar in both wild type and *pma* hind legs (Fig.1B), when aligned relative to the tibial mechanical axis. The *pma* hind feet (Fig.1D) display a number of three-dimensional abnormalities compared to wild type, like human clubfoot it displays supination, (i.e. adduction and inversion of the mid foot and forefoot and plantar-flexion of the ankle and toes). Hypoplasia of the muscles in the antero-lateral (peroneal) compartment of *pma* hind leg was also demonstrated (Fig.1A). To understand how the observed deformities in the *pma* mouse hind foot arise during embryonic development, we followed the process of foot rotation in both wild type and *pma* mutant mice embryos (Fig.1E). Rotation of the hind foot in mouse embryos of wild type strains, CD-1 and C57/Black, occurs from 14 days post-conception onwards with rotation in C57/Black taking longer. Final rotation in some mice is completed post-natally. In *pma* mutants, initiation of rotation is often delayed, and rotation is slower and does not reach completion.

Conclusion: Thus *pma* mutant mouse is a useful animal model for CTEV. If the findings from this study are extrapolated to humans, it would support a long standing hypothesis that CTEV is due to the failure of completion of the normal process of rotation and angulation, historically known as the 'arrested development hypothesis'.